THE FUNCTION OF FATS IN IMMUNE PROCESSES

II. PNEUMOCOCCUS AND STREPTOCOCCUS IMMUNITY

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The work discussed in this paper is a continuation of earlier work[1] in which it was shown that the fats peculiar to certain bacteria and other cells constitute their specific antigens, as shown by complement fixation, antibody production in animals inoculated with the antigens, and by a new specific precipitation reaction in vitro between the fat antigens and their appropriate serum antibodies. This paper deals with the quantity of antibody produced in rabbits from inoculations with the pneumococcus and the streptococcus fat antigens and the amount of protection afforded against the organisms.

In studying the antibodies against the pneumococci obtained in previous experiments[2] on rabbits it was observed that while present in quantity sufficient to respond to the various tests they afforded the animals little or no protection against intravenous injections of pneumococcus in doses fatal to normal animals. For this failure there seemed to be two possible explanations. Either the fat antigens alone did not engender a protective antibody and it was necessary to take the protein of the pneumococcus into consideration, or the fat antigens had not been administered wisely, that is to say in the proper form, or state, or at proper times, or in the proper manner to induce strong protection.

The first of these explanations seemed attractive. We know that antibodies to proteins can be produced in large amounts and that they are protective, but that their specificity is somewhat limited. The studies of Gengou[3] and others showed that the antibody to eggwhite was not able clearly to identify the species of animal from which the antigen was obtained, and that while casein and hemoglobin antibodies always gave precipitates with casein and hemoglobin antigens, they were not able to identify the particular source from which the protein

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[1] Jour. Infec. Dis., 1918, 22, p. 131.
[2] Ibid., 1918, 23, p. 133.
[3] Bordet and Gay: Studies in Immunity, 1909, p. 241.

had been derived. My own studies on the fat antigens showed that their corresponding antibodies possess high specificity, so high in fact as to distinguish clearly between Types I, II and III of the pneumococcus. From these facts it seemed as though the antibodies derived from an attack of an infectious disease or from active immunization with bacteria might depend on the protein fraction of the antigen for their enduring and protective qualities and on the fat fraction for their specificity. Since pneumococcus antibody in whatever way hitherto obtained is invariably small in quantity, of low protective value and yet of high specificity it would appear as if there were some fault in the pneumococcus protein. It was thought then that if there should be substituted for the pneumococcus protein a protein derived from a micro-organism known to produce antibodies of long continuance and high protective value, such for instance as protein from the typhoid bacillus, and if to that protein there should be "grafted," so to speak, the specific fat antigen of the pneumococcus it might be possible with such a combination to produce in animals antibodies having the combined qualities of the respective antigens, namely, protective and specific factors. To test this assumption the following experiments were carried out.

Exper. 1.—A protein was obtained from the defatted typhoid bacillary substance of Vaughan by extraction with distilled water containing a few drops of chloroform at 37 C. for a week, centrifugating the undissolved residue and evaporation of the supernatant and slightly straw-colored liquid to dryness at 20 C. under an air blast. The resulting brittle substance was easily powdered and almost wholly soluble in water and physiologic salt solution. Such solutions contained no coagulable protein but gave strong protein reactions with biuret and Hopkins-Cole reagents, and contained approximately 0.002% free amino-acids as determined by the van Slyke method. This substance was toxic for rabbits, being invariably fatal within 4-8 hours in doses of 0.1 mg. The dose adopted for immunizing purposes was 0.05 mg. which could be repeated with safety as often as every other day. Type I pneumococcus was used, the invariably fatal dose of which for rabbits of 1,800 to 2,000 gm. was found to be 0.05 c c of a 24-hour glucose broth culture, thoroughly shaken. This dose produced death from septicemia within 72 hours.

The pneumococcus artificial general antigen[2] was used for grafting on the protein for purposes of immunization. This consisted of the sodium esters of the fatty acids common to Types I, II and III of the pneumococcus and was administered intravenousy dissolved in salt solution.

Two series of rabbits averaging 1,800 gm. weight were inoculated in duplicate in the following manner: Four rabbits received 4 intravenous inoculations at intervals of 48 hours of 0.05 mg. typhoid protein in 2 c c salt solution. Four received similarly spaced inoculations of 5 mg. of the pneumococcus fat antigen dissolved in 2 c c salt solution; and 4 received similar inoculations of the same quantity of the antigen to which solution there was added 0.05 mg. of typhoid protein.

The rabbits were bled from the heart on the 8th day following the last inoculation and the serums tested for antibodies.

Briefly, the results of the test were as follows: The rabbits injected with typhoid protein gave serums precipitating with the protein antigen solution, 1 : 1,000, and to some extent also with tubercle bacillary protein. Normal horse serum also gave slight precipitates with these antigens whereas pooled normal rabbit serum did not. The serum from the rabbits injected with the pneumococcus fat antigen agglutinated suspensions of pneumococcus type I in equal volumes, and gave precipitates in doses of 0.08 c c with 0.5 c c volumes of the antigen solution containing 0.2 mg. of antigen. The serum from the rabbits injected with the mixed antigens showed antibodies precipitating with the typhoid and the fat antigens severally and combined, but indicating no marked avidity for the combined antigens.

Two days following the bleeding, that is 10 days after the last inoculation, the rabbits received intravenously the invariably fatal dose of pneumococcus culture. All died within the prescribed time of 72 hours, those having received the protein being the first to succumb.

Further trials of the combined antigens on fresh animals showed that in some the time of illness was prolonged and that joints became involved, and it was thought that such instances might show a slight degree of protection probably due to the protein antibody, but the idea was abandoned after similar conditions were observed occasionally to obtain in rabbits injected with fat antigen alone.

From this experiment it was apparent that the assumption on which it was based was erroneous.

It was then determined to ascertain whether the protective power of antibody could be augmented by varying the pneumococcus fat antigen. The fats composing the antigen are of such character that their alkali salts are easily soluble in water and in physiologic salt solution, forming solutions either quite clear or faintly opalescent in the strength employed for inoculation which undergo hydrolysis slowly. The microscope shows no particles in suspension. With the idea of giving greater "surface" to the solutions, or, in other words, of making the antigen a suspension of fairly uniform sized particles more stable and colloidal in character there were presented several expedients, one of which was to produce the desired surface by the addition of suitable amounts of a kation, such for instance as Ca, to the solution of the sodium salts of the antigen, while another was to substitute for the Na another metal which would give fatty salts of less solubility and more easily aggregated by a bivalent kation. The latter idea was chosen and an antigen was made of the lithium salts of the appropriate fatty acids. These salts when dissolved or suspended in Ringer-Locke solution of 0.025% strength gave dense white suspensions, the particles in which, crystalline in character, were visible with a $\times 8$ lens.

Exper. 2.—Four rabbits averaging 1,800-2,000 gm. weight received intravenously at 48-hour intervals 0.5 mg. of the pneumococcus lithium antigen in 2 c c Locke solution. Five days after the last of the injections 2 of the rabbits were bled and their serums shown to contain antibodies by the tests of agglutination of germ suspensions and precipitation with antigen solution. On the 8th day following the last injections the 4 animals together with 2 normal controls were inoculated intravenously with the fatal dose of pneumococcus (Type I) culture. The controls died within 72 hours, 2 of the inoculated animals died within the same period and a third in 96 hours. One rabbit was made ill but recovered.

A second series of 4 rabbits of the same average weight received 6 intravenous inoculations at 48-hour intervals of 0.5 mg. of the lithium antigen in 2 c c of Locke solution. Two days after the last injection, without preliminary bleeding for purpose of testing the serums, the inoculated rabbits and the usual controls were injected intravenously with twice the fatal dose of pneumococcus Type I, that is, 0.1 c c of a 24-hour glucose-broth culture diluted to 1 c c with salt solution. The control animals died on the 3rd day, 2 of the injected rabbits died on the 5th day, while the 2 remaining recovered.

While this series was in progress 4 fresh normal rabbits of even average weight received at the same time with the preceding controls each a single fatal dose of the same culture, which was followed, after 1 hour, by intravenous inoculation of 0.5 mg. of the lithium antigen in 2 c c of Locke solution. This antigen injection was repeated in each animal on the 2 following days, making 3 injections in all. Of these rabbits 2 recovered and 2 died on the 5th day.

It appeared from this experiment that the antibody afforded a considerable measure of protection. It was thought, however, that the antigen would be distinctly improved could it be brought into a more stable colloidal form approaching as closely as possible the state in which the fats might be supposed to exist in the surfaces of the cocci. Previous experience with colloidal cholesterol[2] led to the belief that the state of the fats desired might be approximated through the agency of this substance. Accordingly the pneumococcus fats were converted into the cholesterol esters of the fatty acids[4] and the general type of antigen prepared from these materials. The antigen made from the cholesterol esters is nearly solid at 20 C., and wholly immiscible with water. It is soluble in ether and in hot acetone, from which solutions it forms very stable colloidal solutions on the addition of several volumes of distilled water and evaporation of the solvent. Such solutions of 1 : 1,000 are white by reflected light, and blue-white, faintly translucent and opalescent by transmitted light. A very few of the larger particles are visible as dots under high microscopic power. The addition of electrolytes causes aggregation and increase in the

[4] For methods or Lewkowitsch, Chem. Tech. of Oils, Fats and Waxes, 1913, p. 66 and p. 271.

size of the particles, and the whole of the esters may be flocculated out by suitable concentrations of kation such as Ca. Calcium chlorid added to the colloid in such amount as to make the solution 1 : 10 M. produces aggregates visible with a ×8 lens. Concentration of 1 : 200-1 : 100 M. gives rice to particles best seen in hanging drop under $\frac{1}{12}$ oil immersion varying in size from 0.5-6 mikrons, the majority being about the size of ordinary cocci. They are round, slightly refractile, have lively brownian movement and the resemblance to cocci is striking. Diplococcal forms are not uncommon and occasionally short chains of 3 or 4 are seen. The colloid made from solution in ether is not so homogeneous nor so stable, but contains even greater variety of forms, many of them capsulated. The colloid made from acetone solution remains apparently unchanged for months at room temperature, can be sterilized by boiling without injury and is the more satisfactory form to work with.

Exper. 3.—Eight rabbits of 1,800 gm. average weight received 4 intravenous injections at 48-hour intervals of 0.5 mg. of the pneumococcus cholesterol colloid antigen in 2 c c Locke solution. Two days following the last of the injections 4 rabbits were given twice the fatal dose of pneumococcus I culture intravenously. These animals recovered. Four days after the last injection 2 of the remaining rabbits received intravenously 3 times the fatal dose of pneumococcus culture. One died in 3 days and one recovered. The 2 remaining rabbits received at the same time as the preceding pair 4 times the fatal dose of culture. Both animals died within 72 hours. Synchronous with the injection of 2 control rabbits 4 fresh rabbits of the same average weight received also one fatal dose of culture and, after 1 hour, 0.5 mg. of pneumococcus cholesterol colloid antigen the injections of antigen being repeated daily until 3 doses had been given. The control animals died within the specified time, the 4 inoculated animals recovered. Eight days after the last inoculations the surviving rabbits were bled and all serums were found to give strong precipitation tests with the antigen mentioned under Exper. 1.

From this experiment it was apparent that the antibody induced by the cholesterol colloidal antigen afforded greater protection to the rabbits than those consisting of the Na and Li esters. It is believed that further improvement can be expected as one learns how and in what state the antigen is best administered. It is evident that in an aqueous colloidal solution of an antigen composed of several fat ingredients some of them will be aggregated to the desired proportions by certain concentrations of electrolyte, whereas others will not; in other words, all the ingredients will not be in the same state, so that the probable effect on the animal will be a retardation in the production of certain elements of the antibody. But at the same time it becomes

apparent that in all probability the fluids and cells of the injected animal respond better to certain fats than to others, and from our observations up to the present time it appears that the more stable in physicochemical characters the antigenic fats, the more stable and protective the antibody engendered. The aliphatic series of normal acids from acetic at the lowest end to clupanodonic at the other includes all the antigenic fats found in the organisms and cells thus far studied. At either end of the series are soluble and unstable acids while in the middle are found those most stable and therefore most readily influenced by electrolytes and other factors which regulate aggregation or dispersion. As the antigenic fats of bacterial cells approach either extreme the production of enduring and protective antibody becomes apparently more difficult, while an organism whose fats occupy middle ground develops strong antibody. The typhoid group of organisms well represents the stable type and the pneumococcus group one of the unstable.

The conditions of the foregoing experiments were purposely made somewhat severe in order to place the pneumococcus antigen under strict test. Type I pneumococcus was chosen because of the regularity of infectiousness for rabbits, the brevity of the course of the infection and the smallness of the fatal dose. The "blanket" pneumococcus antigen was selected in order to determine whether it would afford protection against the most infectious of the types, although it does not strictly represent the true antigen for Type I. It will be recalled[2] that while the general antigen covers Types I, II and III, its ingredients form the average fat antigen content of all 3 and is not the exact antigen for Type I, but is the exact one for Type II, the 3 types having been shown to contain the same fat substances only in slightly different proportions. From the results of the experiments it seems justifiable to infer that if the blanket antigen affords a degree of protection, as it has been shown to do, against the most virulent member of the group it will induce protection against the other less virulent members.

While the results of the experiment with bacterial protein are of course inconclusive, nevertheless, the impression gained from the work up to the present moment has become progressively stronger that protein immunization and specific cell immunization may be two quite distinct processes, and that the rôle of protein in cell immunity is probably so slight as to be almost if not wholly negligible. With the

protein immune process occur the factors of sensitization, toxicity, specificity for type only and not for species, while with fat immunization there is no sensitization and no toxicity, but marked species specificity. Rabbits immunized with fat antigens never show sensitization on reinjection after a lapse of time, nor do toxic symptoms occur except in the manner of anaphylaxis which may be induced by the "surface" of the antigen suspensions in the same way as by the surface of agar, starch or inulin suspensions. In the preceding paper[4] there were mentioned instances of undoubted sensitization in rabbits following reinjection with fats. The sudden deaths in these animals may have been and probably were due to anaphylaxis from surface action.

THE STREPTOCOCCUS ANTIGEN

The cultures of streptococcus used consisted of 6 strains each of S. viridans and S. hemolyticus, all of which had been isolated from cases of acute infections in men a short time prior to this study. The fats of the combined strains of organisms were obtained in a manner identical with that employed in the case of the pneumococcus antigen,[2] examined, and from the data acquired there was prepared an artificial streptococcus antigen of the general type.

By the methods of precipitation and agglutination employed as before described, the antigen was tested against supposed antistreptococcus serums prepared by the commercial houses and purchased in the market. Briefly, one of two conclusions was to be drawn from the tests. Either the antigen was faulty or the serums contained no antibody. Inasmuch as the serums were found to possess no powers of agglutinating streptococci beyond that shown by normal horse serum or physiologic salt solution, and gave no precipitates with the antigen, and as the antigen represented the average of several close fat determinations it was concluded that the fault did not lie with the antigen. If, then, it could be shown that injections of the fat antigen into rabbits would produce antibody with power of agglutination over the cocci and of precipitation of the antigen the conclusion that the antigen had been approximated would be justified. Accordingly, the injections were carried out.

Exper. 4.—As representative of the method employed the protocol of one series of animals is noted here. Four rabbits of 2,000 gm. average weight received 5 intravenous inoculations of 0.5 mg. of the streptococcus cholesterol colloid antigen in 2 c c of Locke solution. Three days after the last injection

the rabbits were bled and their serums tested, two for agglutinating power and all for precipitation.

The suspension of streptococcus was made as follows: Twenty-four-hour glucose-broth cultures of the various strains of streptococci were combined and centrifugated, the clear broth pipetted off and the residue suspended in 0.6% salt solution. This suspension was then thoroughly shaken and again centrifugated for a short time at moderate speed to remove clumps. The resulting emulsion was fairly thick and homogeneous. The serum dilutions were 1:10, 1:50 and 1:100. The test tubes after mixing were placed in the icebox for 8 hours.

Rabbit 1 serum showed complete agglutination in dilution of 1:10, partial in 1:50 and slight in 1:100.

Rabbit 2 serum showed partial agglutination in dilution in 1:10, less in 1:50, and none in 1:100.

Normal rabbit serum produced no agglutination in the same dilutions. The control suspension was not agglutinated.

The antigen used consisted of an alcoholic solution of the sodium salts of the fatty acids of the streptococcus in such strength that 1 c c contained 2 mg. of the salts. To 1 c c of this solution there was added 0.6 c c of a 1% alcoholic solution of cholesterol. The dose of this completed antigen, 0.1 c c, therefore contained 0.125 mg. of fat and 0.375 mg. of cholesterol. The tubes were placed in the icebox for 6-8 hours.

TABLE 1

PRECIPITATION

With 0.5 c c Salt Solution plus 0.1 c c Antigen

Serum 1, 0.08 c c = partial precipitation	Serum 3, 0.24 c c = complete precipitation
Serum 1, 0.24 c c = complete precipitation	Serum 4, 0.08 c c = partial precipitation
Serum 2, 0.08 c c = complete precipitation	Serum 4, 0.24 c c = complete precipitation
Serum 2, 0.24 c c = partial precipitation	Salt solution, 0.08 c c = no precipitation
Serum 3, 0.08 c c = complete precipitation	Nor. rabbit serum, 0.24 c c = no precipitation

From this experiment it was concluded that antibodies against the streptococcus were produced in the serums of the injected animals.

Owing to difficulties the protective power of the antibodies against infection by streptococcus was not determined in rabbits. The strains of organisms used were found to possess low virulence. Large doses were necessary to infect, no uniform dose produced like degrees of infection and the infection itself was of the chronic type, many of the rabbits recovering. By means of the precipitation test it was observed that the serums of some rabbits apparently contain a slight amount of antibody against the streptococcus, but no serum was found to hold any amount comparable to that in the serums of the inoculated animals.

In order to test whether rabbits having no streptococcus antibody, or slight amounts only, would develop it following inoculations of the antigen in another form and in a different manner, 3 selected rabbits were given 4 subcutaneous injections, 2 days apart, of 0.5 mg. of the

streptococcus antigen composed of the lithium salts. On the 3rd day following the last injection the rabbits were bled. Table 2 shows the presence of antibodies in greater or less amount.

TABLE 2

PRECIPITATION

With 0.5 c c of 0.6% Salt Solution plus 0.1 c c Antigen

Serum 1, 0.08 c c = no precipitation	Serum 3, 0.08 c c = partial precipitation
Serum 1, 0.16 c c = partial precipitation	Serum 3, 0.16 c c = partial precipitation
Serum 1, 0.24 c c = complete precipitation	Serum 3, 0.24 c c = partial precipitation
Serum 2, 0.08 c c = partial precipitation	Serum nor. rabbit, 0.24 c c = no precipitation
Serum 2, 0.16 c c = partial precipitation	Salt solution, 0.08 c c = no precipitation
Serum 2, 0.24 c c = complete precipitation	(Icebox 8 hours)

The availability of subcutaneous inoculations of fat antigens for the production of immunity in man is illustrated by the following experiment.

Exper. 5.—Four men between the ages of 20 and 40 years were bled and their serums shown by complement fixation, agglutination and precipitation tests to contain no antibodies against, respectively, the typhoid bacillus, the pneumococcus, the streptococcus and the treponema pallida.

1. Received 3 subcutaneous injections of 5 mg. of pneumococcus cholesterol colloid antigen in 2 c c salt solution containing $CaCl_2$ 1:100 M. at intervals of 2 days.

2. Received 4 similar injections of a like amount of streptococcus colloid antigen at intervals of 4 days.

3. Received 4 similar injections of typhoid antigen.

4. Received 4 similar injections of syphilis antigen.

Five days after the last injection the men were bled again. Their serums gave precipitates with their respective antigens but not with crossed antigens. The serum of the man last mentioned also gave a positive syphilitic complement fixation test. The serum of the 3rd man agglutinated suspensions of B. typhosus, parat. A, parat. B. and B. coli in dilutions of 1:200.

Granted that animals develop antibodies against certain combinations of fats which represent the antigenic factors in cells it should be possible to induce antibody in rabbits by injection of a single, isolated fat, in which case the interaction of antigen and antibody in precipitation would afford a biologic test for the fat in question.

Exper. 6.—Two rabbits weighing approximately 2,000 gm. were bled from the heart and their serums tested for precipitation with an antigen composed of an alcoholic solution of sodium oleate containing 2 mg. of the salt in 1 c c, to which was added a 1% solution of cholesterol (alcoholic) in the proportion of 0.6 c c for each 1 c c of the oleate solution. The serums caused no precipitation with 0.1 c c of the antigen in 0.5 c c of salt solution in doses as high as 0.32 c c. The salt of oleic acid was chosen as being one of the most difficult in the series with which to produce antibody owing to its solu-

bility and perhaps also to its slightly unsaturated character. Another reason for the choice was because of its common occurrence as such or in the form of the glycerid in animal tissues.

The 2 rabbits then received 4 intravenous injections of 0.5 mg. of lithium oleate in 2 c c of Ringer solution, the second injection being given after an interval of 4 days and the following injections at intervals of 2 days. Two days after the last inoculation the rabbits were bled and their serums tested with the sodium oleate antigen as follows.

TABLE 3

PRECIPITATION TEST

With 0.5 c c of 0.6% Salt Solution Plus 0.1 c c Antigen,

Serum 1, 0.12 c c = partial precipitation	Serum 2, 0.08 c c = partial precipitation
Serum 1, 0.20 c c = complete precipitation	Serum 2, 0.20 c c = partial precipitation
Serum 1, 0.32 c c = complete precipitation	Serum 2, 0.32 c c = partial precipitation
Serum nor. rabbit, 0.32 c c = no precipitation	Salt solution, 0.08 c c = no precipitation

(Icebox 6-8 hours)

One week later the 2 rabbits received 2 intravenous injections, with an interval of 2 days, of 2 c c of cholesterol oleate colloid antigen containing 0.5 mg. of the ester. Three days after the last injection the rabbits were again bled. Their serums showed practically no increase in antibody. Without further injections the animals were bled again after the expiration of 1 week. The results of the precipitation test with the sodium oleate antigen, together with similar antigens of sodium linoleate, and sodium palmitate, acids respectively above and below oleic, are shown in Table 4.

TABLE 4

PRECIPITATION TEST

With 0.5 c c Salt Solution Plus 0.1 c c Oleic Antigen; 0.1 c c Linoleate Antigen; Palmitate Antigen,

Serum 1, 0.08 c c = complete precipitation	Serum 2, 0.24 c c = complete precipitation
Serum 1, 0.24 c c = complete precipitation	Serum 1, 0.24 c c = complete precipitation
Serum 2, 0.08 c c = complete precipitation	Nor. rabbit serum, 0.24 c c = no precipitation
Serum 2, 0.24 c c = complete precipitation	Antistrep. ser., 0.24 c c = no precipitation

No precipitations with palmitate or linoleate antigens.

From this experiment one may tentatively conclude that rabbits may be immunized against an isolated fat, and that their serums will react with precipitation to the fat in the proper antigen, but not to fats closely related but with different characters and molecular weights. It will be observed in the table that a pneumococcus antiserum gave a precipitate with the sodium oleate antigen. This phenomenon might be expected since oleic acid is an important constituent of the pneumococcus fats, and is quite comparable if not identical in causation with that of group agglutination and complement fixation. It has been a matter of occasional observation in the course of this work that when one fat is a prominent ingredient of 2 antigens the antiserums to those antigens will give partial precipitation with the related antigen

but not with antigens in which the fat plays a minor part. Certain unfinished experiments, however, seem to indicate that an immune serum against an antigen composed of several fats will give precipitates more or less complete with the individual fats composing it. Thus it has been observed that antityphoid serum will precipitate not only with the typhoid antigen,[2] but also with solutions of each of its several components, probably because the latter exist almost in equal parts in the combination.

The apparent power of an immune serum to precipitate with the individual components of a fat antigen complex led to the inquiry whether it were possible by such means to approximate qualitatively the fatty constituents of all the fatty antigens in use, in which case the results would be a check on the chemical analysis of the cells. This has not been possible in all instances thus far. Where a constituent of an antigen exists in small amount only, an immune serum, while precipitating with the fat as part of the antigen complex, does not precipitate with the individual fat in the ordinary concentrations as used in antigen-salt solution-serum mixtures. It appears that there is required different proportions of electrolyte, fat and serum. These proportions have not yet been worked out, but from the inquiry some interesting suggestions have risen. For instance, working with pooled syphilitic serums the results of precipitation with individual fats gave qualitative results corresponding very closely with the analysis of the syphilitic antigen; and while an analysis of human red cells has not been attempted a qualitative suggestion was obtained from immune rabbit serum as shown in Table 5.

TABLE 5

RESULTS OF PRECIPITATION WITH INDIVIDUAL FATS

Fat Antigens, Alcoholic Solution in 0.75 c c 0.6% Salt Solution	Total Quantity of Mixtures 0.75 c c	
	Normal Rabbit Serum, 0.2 c c	Rabbit-Human Serum, 0.2 c c
Sodium Caprate 0.06 gm. No precipitation	No precipitation	Partial precipitation
Sodium Laurate 0.06 gm. No precipitation	No precipitation	Partial precipitation
Sodium Myristate 0.06 gm. No precipitation	Partial precipitation	Complete precipitation
Sodium Palmitate 0.05 gm. No precipitation	No precipitation	Partial precipitation
Sodium Stearate 0.06 gm. No precipitation	No precipitation	Partial precipitation
Sodium Cerotate 0.07 gm. No precipitation	No precipitation	No precipitation
Sodium Melyssate 0.07 gm. No precipitation	No precipitation	Complete precipitation
Sodium Oleate 0.07 gm. No precipitation	No precipitation	Complete precipitation
Sodium Linolate 0.07 gm. No precipitation	No precipitation	Complete precipitation
Sodium Clupanodinic 0.07 gm. No precipitation	No precipitation	Complete precipitation

(Icebox 8 hours.)

These reactions suggest a highly complex composition for human red cells which no doubt is not far from the truth since they of all animal cells must be "immune" to the widest range of fats.

The reaction of precipitation, or agglutination, between immune serums and fats must be regarded as largely tentative. It requires the balance of ingredients and delicacy of manipulation as correct as in complement fixation, on which indeed it is in part founded, and the result of a large number of experiments must be observed before final deductions may be drawn. Particularly troublesome are spontaneous precipitations of antigen and salt solution controls which occur when proportions or measurements are incorrect. Some individual fat controls demand stabilization with a dispersive colloid such, for instance, as 0.01 c c or 0.02 c c of normal rabbit or human serum, a procedure which is entirely permissible.

The facts brought out in the work on the functions of antigenic fats in immunity lead one to believe that such antigens are destined to play an important part not only in active immunization of animals and man as a prophylactic measure, but also in the treatment of infections. They have to commend them their purity, the dosage by weight, the absence of toxicity, the ease and safety of either subcutaneous or intravenous administration.

This form of treatment would appear to be particularly applicable to the types of infections of acute and often fatal character and of brief duration where antibody production is invariably slight or absent altogether, and where toxemia is the dominant symptom, such types being represented by pneumococcus, streptococcus and meningococcus infections. In infections also of a more prolonged course such as typhoid and paratyphoid fevers, even when antibody is known to be present in the serum, as shown by agglutination tests, at the same time with the antigenic micro-organism, the fat antigen would be presumed to be beneficial both by increasing the antibody production and by furnishing a nidus round which the antigen-antibody aggregate might form and lead to the absorption of complement.